Abstract: The present invention provides fusion proteins expressed by cancer cells, antibodies and other antigen-binding agents that specifically bind to the fusion proteins, and compositions and methods for using the antibodies and other antigen-binding agents to detect, characterize, and treat cancer.
SCNN1A/TNFRSF1A FUSION PROTEINS IN CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/360,381, filed June 30, 2010 and U.S. Provisional Application No. 61/501,080, filed June 24, 2011, which are hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] This invention relates to novel fusion proteins expressed by ovarian and other cancer cells, and to compositions and methods for detecting, characterizing, and treating ovarian and other cancers.

BACKGROUND OF THE INVENTION

[0003] Cancer is the second-leading cause of death in the U.S. In 2011, the American Cancer Society projects 1,596,670 new cancer cases and 571,950 deaths from cancer. Ovarian cancer is the ninth most common cancer in women and the fifth most common cause of cancer death in women in the U.S. Ovarian cancer includes those tumors that arise from various tissue types of the ovary. Tumors are classified according to the cell type from which they originate, including three main types of tumors: epithelial tumors (including the most common serous subtype), germ cell tumors, and stromal cell tumors. Ovarian cancer is difficult to diagnose at an early stage due to its non-specific symptoms and rapid rate of metastasis. The majority of ovarian cancer cases are therefore diagnosed at a late stage, leading to low survival rates. There is a high unmet need for better methods of detecting and treating ovarian cancer, as well as lung, bladder, thyroid, and other tumor types.

SUMMARY OF THE INVENTION

[0004] The present invention provides novel fusion proteins, including fusion junction peptides that are overexpressed by ovarian cancer cells and other cancer cells as compared to normal cells. The invention provides the fusion proteins SEQ ID NO: 4 and SEQ ID NO: 6 as well as fragments thereof.

[0005] In some embodiments the fusion proteins comprise polypeptide fragments including a 12/2 form of the junction peptide, e.g. comprising amino acids 539-548 of SEQ ID
NO: 4, amino acids 535-553 of SEQ ID NO: 4, amino acids 530-559 of SEQ ID NO: 4, amino acids 524-563 of SEQ ID NO: 4, or larger fragments of SEQ ID NO: 4 including amino acids 535-553 of SEQ ID NO: 4. In some embodiments the fusion proteins comprise polypeptide fragments including a 12/2 form of the junction peptide, e.g. comprising amino acids 391-548 of SEQ ID NO: 4, amino acids 386-548 of SEQ ID NO: 4, amino acids 381-548 of SEQ ID NO: 4, or larger fragments of SEQ ID NO: 4 including amino acids 391-548 of SEQ ID NO: 4.

[0006]
Some aspects of the invention provide a contiguous fragment of SEQ ID NO: 4 including at least amino acids x-553, wherein in some embodiments x is a residue selected from amino acids 1-391 and in some embodiments x is a residue selected from amino acids 108-391. In some embodiments the fusion proteins comprise amino acids 108-741 of SEQ ID NO: 4.

[0007]
In some embodiments the fusion proteins comprise polypeptide fragments including a 13/2 form of the junction peptide, e.g. comprising amino acids 600-609 of SEQ ID NO: 6, amino acids 596-614 of SEQ ID NO: 6, amino acids 591-620 of SEQ ID NO: 6, amino acids 585-624 of SEQ ID NO: 6, or larger fragments of SEQ ID NO: 6 including amino acids 596-614 of SEQ ID NO: 6. In some embodiments the fusion proteins comprise amino acids 108-802 of SEQ ID NO: 6.

[0008]

[0009]
The present invention provides nucleic acids encoding the novel fusion proteins, fragments thereof, and also probes and primers used to amplify and detect nucleic acids encoding the novel fusion proteins. The nucleic acids may be double stranded or single stranded, the nucleic acids may be DNA, RNA, or artificial variants thereof. In some embodiments the nucleic acids encode polypeptides comprising or consisting of SEQ ID NO: 4 or SEQ ID NO: 6.

[0010]


[0012] Some aspects of the invention provide a nucleic acid encoding a contiguous fragment of SEQ ID NO: 4 including at least amino acids x-553, wherein in some embodiments x is a residue selected from amino acids 1-391 and in some embodiments x is a residue selected from amino acids 108-391.

[0013] Some aspects of the invention provide primer pairs for amplifying nucleic acids encoding the 12/2 or 13/2 junction, e.g. the primer pair SEQ ID NO: 7 and SEQ ID NO: 8 or the primer pair SEQ ID NO: 10 and SEQ ID NO: 11. The primers can be any length, for example, 15, 20, 25, 30, or more nucleotides in length, in which one member of the primer pair hybridizes to a nucleic acid encoding the SCNN1A portion of the fusion sequence and one member of the primer pair hybridizes to a nucleic acid encoding the TNFRSF1A portion of the fusion sequence. Some aspects of the invention provide probes for detecting nucleic acids encoding the 12/2 or 13/2 fusion junction, e.g. SEQ ID NO: 9 or SEQ ID NO: 12. In some embodiments the probes are labeled with a fluorescent or isotope label. The probes can be any length, for example, 15, 20, 25, 30, or more nucleotides in length, in which the probe recognizes the SCNN1A 12/2 TNFRSF1A fusion junction or the 13/2 TNFRSF1A fusion junction.

[0014] Also provided are vectors comprising the nucleic acids of the invention. In one embodiment the vector is an expression vector. Also provided is a host cell comprising the vector.

[0015] The invention provides recombinant host cells that express the 12/2 or 13/2 fusion proteins or fragments thereof on the cell surface.

[0016] In some embodiments the invention provides antigen-binding agents including antibodies that specifically bind to the fusion junction peptides and fusion proteins, the antigen
binding agents preferably having a greater affinity for the fusion proteins than for either of the fusion partners that make up the fusion protein.

[0017] The antibodies of the invention may be monoclonal or polyclonal antibodies. In some embodiments the antibodies are chimeric, humanized, or human antibodies. In some embodiments the antibodies are single chain antibodies or Fab fragments.

[0018] In some aspects the invention provides an isolated antigen binding agent that specifically binds to a SCNNIA /TNFRSFIA fusion protein. In some preferred embodiments the binding agent is a monoclonal antibody. In some aspects the invention provides an isolated monoclonal antibody or other antigen binding agent that specifically binds to a SCNNIA /TNFRSFIA fusion protein, wherein the antigen binding agent specifically binds to a SCNNIA /TNFRSFIA fusion protein selected from: (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1, and (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2.

[0019] In some aspects the invention provides a monoclonal antibody wherein the antibody binds a SCNNIA /TNFRSFIA fusion protein with an affinity less than 1 nM and specifically binds a SCNNIA /TNFRSFIA fusion protein with at least 100-fold higher affinity than it binds either SCNNIA or TNFRSFIA.


[0021] Some aspects of the invention provide an antibody or other binding agent that specifically binds a fragment of SEQ ID NO: 4 including at least amino acids x-553, wherein in some embodiments x is a residue selected from amino acids 1-391 and in some embodiments x is a residue selected from amino acids 108-391.

[0022] In some embodiments the antibody or other binding agent specifically binds to the fusion proteins SEQ ID NO: 4, SEQ ID NO: 6, or to fragments thereof, expressed on the surface of a recombinant cell.

[0023] Also provided is a hybridoma capable of producing the antibodies of the present invention. Also provided is a method of making the antibodies or other antigen binding agents
comprising culturing a host cell under conditions that allow it to express the antigen binding agent.

[0024] The invention provides methods of making antibodies that can be used for detecting, characterizing, and treating tumors comprising immunizing an animal with a polypeptide selected from SEQ ID NO: 4 or a fragment thereof comprising the sequence SEQ ID NO: 1, SEQ ID NO: 6 or a fragment thereof comprising the sequence SEQ ID NO: 2, amino acids 539-548 of SEQ ID NO: 4, amino acids 535-553 of SEQ ID NO: 4, amino acids 530-559 of SEQ ID NO: 4, amino acids 524-563 of SEQ ID NO: 4, amino acids 391-548 of SEQ ID NO: 4, amino acids 386-548 of SEQ ID NO: 4, amino acids 108-741 of SEQ ID NO: 4, amino acids 600-609 of SEQ ID NO: 6, amino acids 596-614 of SEQ ID NO: 6, amino acids 591-620 of SEQ ID NO: 6, amino acids 585-624 of SEQ ID NO: 6, and amino acids 108-802 of SEQ ID NO: 6. In some embodiments the polypeptide used to immunize the animal is expressed on the surface of a recombinant cell. In some embodiments the resulting antibodies are isolated using standard methods known in the art. In some embodiments the animal expresses human antibodies.

[0025] The invention provides methods of making antibodies that can be used for detecting, characterizing, and treating tumors comprising screening a library of antibodies expressed on phage, phagemids, ribosomes, or other particles with a polypeptide selected from SEQ ID NO: 4 or a fragment thereof comprising the sequence SEQ ID NO: 1, SEQ ID NO: 6 or a fragment thereof comprising the sequence SEQ ID NO: 2, amino acids 539-548 of SEQ ID NO: 4, amino acids 535-553 of SEQ ID NO: 4, amino acids 530-559 of SEQ ID NO: 4, amino acids 524-563 of SEQ ID NO: 4, amino acids 391-548 of SEQ ID NO: 4, amino acids 386-548 of SEQ ID NO: 4, amino acids 108-741 of SEQ ID NO: 4, amino acids 600-609 of SEQ ID NO: 6, amino acids 596-614 of SEQ ID NO: 6, amino acids 591-620 of SEQ ID NO: 6, amino acids 585-624 of SEQ ID NO: 6, and amino acids 108-802 of SEQ ID NO: 6. In some embodiments the polypeptide used to screen the library is expressed on the surface of a recombinant cell. In some embodiments the resulting antibodies are isolated using standard methods known in the art. In some embodiments the library is a library of human antibodies.

[0026] Also provided are isolated nucleic acid molecules comprising a polynucleotide sequence encoding the light chain variable domain, the heavy chain variable domain, or both, of the antibodies or other antigen binding agents of the invention. In one embodiment, the polynucleotide comprises a light chain variable sequence, and a heavy chain variable sequence.
In some aspects the invention provides bispecific antibodies or other binding agents in which one antigen-binding site binds an epitope on SCNN1A and one antigen-binding site binds an epitope on TNFRSF1A. In some aspects of the invention the bispecific binding agent is an antibody that specifically binds to a SCNN1A/TNFRSF1A fusion protein selected from: (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1, and (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2.

In some aspects the invention provides antibodies with enhanced effector functions. In other aspects the invention provides antibodies conjugated to a toxin or other therapeutic agent. In some aspects of the invention the toxin or other therapeutic agent is joined to the antibody by means of a cleavable or non-cleavable linker. In some aspects the toxin or other therapeutic agent is an auristatin or maytansinoid.

Also provided is a pharmaceutical composition comprising the antibodies or other antigen binding proteins of the present invention. In one embodiment the pharmaceutical composition comprises a human antibody.

In some embodiments the invention provides methods useful to detect and characterize ovarian, bladder, lung, thyroid, and other types of tumors. Some aspects of the invention comprise detecting expression of a fusion protein according to the invention in cells from the tumor. In some embodiments the fusion protein detected is selected from: (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1, and (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2. Some embodiments comprise detecting a polypeptide comprising amino acids 539-548 of SEQ ID NO: 4, amino acids 535-553 of SEQ ID NO: 4, amino acids 530-559 of SEQ ID NO: 4, amino acids 524-563 of SEQ ID NO: 4, amino acids 391-548 of SEQ ID NO: 4, amino acids 386-548 of SEQ ID NO: 4, amino acids 381-548 of SEQ ID NO: 4, amino acids 108-741 of SEQ ID NO: 4, amino acids 600-609 of SEQ ID NO: 6, amino acids 596-614 of SEQ ID NO: 6, amino acids 591-620 of SEQ ID NO: 6, amino acids 585-624 of SEQ ID NO: 6, or amino acids 108-802 of SEQ ID NO: 6. Some embodiments comprise detecting polypeptides consisting of amino acids 539-548 of SEQ ID NO: 4, amino acids 535-553 of SEQ ID NO: 4, amino acids 530-559 of SEQ ID NO: 4, amino acids 524-563 of SEQ ID NO: 4, amino acids 391-548 of SEQ ID NO: 4, amino acids 386-548 of SEQ ID NO: 4, amino acids 381-548 of SEQ ID NO: 4, amino acids 108-741 of SEQ ID NO: 4, amino acids 600-609 of SEQ ID NO: 6, amino acids 596-614 of SEQ ID NO: 6, amino acids 591-620 of SEQ ID NO: 6, amino acids 585-624 of SEQ ID NO: 6, or amino acids 108-802 of SEQ ID NO: 6. In some embodiments the fusion protein detected is a contiguous fragment of SEQ ID NO: 4 including at least amino acids x-553, wherein in some
embodiments x is a residue selected from amino acids 1-391 and in some embodiments x is a residue selected from amino acids 108-391.

[0031] In some aspects of the invention expression of the fusion protein is detected at the level of RNA. In other aspects of the invention expression of the fusion protein is detected at the level of protein. In some aspects of the invention expression of the fusion protein is detected through the use of an antibody that specifically binds the fusion protein. The invention includes embodiments in which nucleic acids encoding the fusion proteins are amplified using a primer pair in which one member of the primer pair hybridizes to the SCNN1A portion of the fusion sequence and one member of the primer pair hybridizes to the TNFRSF1A portion of the fusion sequence. In some embodiments the primer pair comprises SEQ ID NO: 7 and SEQ ID NO: 8 or the primer pair comprises SEQ ID NO: 10 and SEQ ID NO: 11. The invention includes embodiments in which nucleic acids encoding the fusion proteins are detected using a probe for the fusion protein junction. In some embodiments the probe comprises SEQ ID NO: 9 or SEQ ID NO: 12.

[0032] In some embodiments, antibodies or other antigen binding agents of the invention are useful to treat ovarian, bladder, lung, thyroid, and other cancers or for preparing a medicament for use in treating ovarian, bladder, lung, thyroid, and other cancers. The invention includes a method of inhibiting proliferation of cells expressing a SCNN1A /TNFRSF1A fusion protein comprising contacting the cells with a composition comprising an antigen binding agent or bispecific antibody that specifically binds to a SCNN1A /TNFRSF1A fusion protein. In some aspects the antigen binding agent is an antibody according to the invention. Some aspects of the invention are directed to a method of treating a patient with cancer, comprising administering a composition comprising an antigen binding agent or bispecific antibody that specifically binds to a SCNN1A /TNFRSF1A fusion protein. Some aspects of the invention are directed to a method of preparing a medicament for use in treating a patient with cancer, comprising a composition comprising an antigen binding agent or bispecific antibody that specifically binds to a SCNN1A /TNFRSF1A fusion protein. In some aspects the antigen binding agent is an antibody according to the invention.

[0033] In some aspects of the invention a patient is treated with an antibody or other antigen binding agent that specifically binds to a SCNN1A /TNFRSF1A fusion protein selected from: (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1, and (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2.

[0034] In some embodiments a patient is treated with an antigen binding agent that specifically binds to a SCNN1A /TNFRSF1A fusion protein comprising amino acids 539-548
of SEQ ID NO: 4, amino acids 535-553 of SEQ ID NO: 4, amino acids 530-559 of SEQ ID NO: 4, amino acids 524-563 of SEQ ID NO: 4, amino acids 381-548 of SEQ ID NO: 4, amino acids 391-548 of SEQ ID NO: 4, amino acids 381-548 of SEQ ID NO: 4, amino acids 391-548 of SEQ ID NO: 4, amino acids 591-614 of SEQ ID NO: 6, amino acids 591-620 of SEQ ID NO: 6, amino acids 585-624 of SEQ ID NO: 6, or amino acids 108-802 of SEQ ID NO: 6. In some embodiments a patient is treated with an antigen binding agent that specifically binds to a SCNN1A/TNFRSF1A fusion protein consisting of amino acids 539-548 of SEQ ID NO: 4, amino acids 535-553 of SEQ ID NO: 4, amino acids 530-559 of SEQ ID NO: 4, amino acids 524-563 of SEQ ID NO: 4, amino acids 381-548 of SEQ ID NO: 4, amino acids 108-741 of SEQ ID NO: 4, amino acids 600-609 of SEQ ID NO: 6, amino acids 591-614 of SEQ ID NO: 6, amino acids 591-620 of SEQ ID NO: 6, amino acids 585-624 of SEQ ID NO: 6, or amino acids 108-802 of SEQ ID NO: 6. In some embodiments a patient is treated with an antigen binding agent that specifically binds to a SCNN1A /TNFRSF1A fusion protein that is a contiguous fragment of SEQ ID NO: 4 including at least amino acids x-553, wherein in some embodiments x is a residue selected from amino acids 1-391 and in some embodiments x is a residue selected from amino acids 108-391.

[0035] In some aspects of the invention the patient is treated with a monoclonal antibody wherein the antibody binds a SCNN1A/TNFRSF1A fusion protein with an affinity less than 1 nM and specifically binds a SCNN1A/TNFRSF1A fusion protein with at least 100-fold higher affinity than it binds either SCNN1A or TNFRSF1A.

[0036] In some aspects of the invention the patient is treated with antibodies having enhanced effector functions. In some aspects of the invention the patient is treated with antibodies conjugated to a toxin or other therapeutic agent. In some aspects of the invention the therapeutic agent is joined to the antibody by means of a cleavable or non-cleavable linker. In some aspects of the invention the therapeutic agent is an auristatin or maytansinoid.

[0037] In some aspects of the invention treatment is administered after detection of the expression of a SCNN1A/TNFRSF1A fusion protein in cells from the cancer. In some embodiments the invention provides a method of treating a patient with cancer comprising the steps of: (a) detecting expression of a SCNN1A/TNFRSF1A fusion protein in cells from the cancer, and (b) administering an antibody specifically binds to a SCNN1A/TNFRSF1A fusion protein. In some embodiments the antibody specifically binds to a SCNN1A/TNFRSF1A fusion protein selected from: (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence
SEQ ID NO: 1, and (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] Figure 1 shows SCNN1A 12/2 TNFRSF1A transcript expression as measured by Quantitative PCR in normal tissue samples and in tumor samples. Relative expression (2^e^(-dCt)) for each tissue sample (A) has been normalized to the expression of a housekeeping gene (beta-actin) run on the same plate.

[0039] Figure 2 shows SCNN1A 13/2 TNFRSF1A transcript expression as measured by Quantitative PCR in normal tissue samples and in tumor samples. Relative expression (2^e^(-dCt)) for each tissue sample (A) has been normalized to the expression of a housekeeping gene (beta-actin) run on the same plate.

DETAILED DESCRIPTION OF THE INVENTION

[0040] The present invention identifies novel fusion transcripts expressed by ovarian cancer cells and other tumor type cancer cells and provides antigen binding agents, including antibodies, antibody fragments, and antibody derivatives that specifically bind to the corresponding fusion junction peptides and fusion proteins. The antigen binding agents are useful for detecting, characterizing, and treating ovarian tumors and other cancers.

[0041] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al, Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and
Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

DEFINITIONS

[0042] The term "isolated molecule" (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be "isolated" from its naturally associated components. A molecule also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[0043] The term "antigen binding agent" refers to a natural or non-natural molecule, preferably a proteinaceous molecule, that specifically binds to a target. The term "specific binding" or "specifically binds" refers to the ability of an antigen binding agent to bind to a target with greater affinity (strength of binding) than it binds to a non-target. In certain embodiments, specific binding refers to binding to a target with an affinity that is at least 10, 50, 100, 250, 500, or 1000 fold greater than the affinity for a non-target. In certain embodiments, affinity is determined by an affinity ELISA assay, by a BIAcore assay, by a kinetic method, or by an equilibrium/solution method. Affinity can be expressed in terms of the dissociation constant Kd.
Examples of antigen binding agents include, but are not limited to proteins, peptides, nucleic acids, carbohydrates, lipids, and small molecule compounds. In some preferred embodiments of the invention the antigen binding agent is an antigen binding protein; in some preferred embodiments of the invention the antigen binding agent is an antibody.

An "antigen binding protein" is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (e.g., an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al, 2003, Proteins: Structure, Function, and Bioinformatics, Volume 53, Issue 1:121-129; Roque et al, 2004, Biotechnol. Prog. 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

Antigen binding proteins further include peptibodies. The term "peptibody" refers to a molecule comprising an antibody Fc domain attached to at least one peptide. The production of peptibodies is generally described in PCT publication WO 00/24782, published May 4, 2000. Peptibodies according to the invention include those in which the peptide antigen-binding site portion(s) bind to the 12/2 or 13/2 fusion proteins, peptibodies in which one peptide recognizes SCNN1A and another peptide recognizes TNFRSF1A, and peptibodies in which one peptide recognizes the fusion protein (e.g., SCNN1A 12/2 TNFRSF1A or SCNN1A 13/2 TNFRSF1A) and another peptide recognizes TNFRSF1A or SCNN1A.

Antigen binding proteins further include nonimmunoglobulin avidity multimers or "avimers," which are multidomain proteins derived from the A-domains as found in various cell surface receptors. Avimers can be generated by the sequential selection of individual binding domains, each of which recognize a different epitope, and can therefore bind multiple sites on a target or even multiple targets. See, e.g., Silverman, J. et al. Nat. Biotechnol. 23, 1556-1561 (2005).
An "epitope" is that portion of the antigen that an antigen binding agent recognizes.

An "antigen binding site" is the portion of an antigen binding agent that contains amino acid residues or other moieties that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

An antigen binding protein may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For example, a naturally occurring human immunoglobulin typically has two identical binding sites, while a "bispecific" or "bifunctional" antibody may have two different binding sites.

An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) . The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

Naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. Intact antibodies include polyclonal, monoclonal, chimeric, humanized or fully human having full length heavy and light chains.
The terms "peptide," "polypeptide," and "protein" each refer to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, e.g., native and artificial proteins, protein fragments and polypeptide analogs such as muteins, variants, and fusion proteins of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins.

The terms "polynucleotide" and "nucleic acid" are used interchangeably throughout and include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs (e.g., peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding an antibody, or a fragment, derivative, mutein, or variant thereof, of the invention. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector.

A "vector" is a nucleic acid that can be used to introduce another nucleic acid linked to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double stranded DNA molecule into which additional nucleic acid segments can be ligated. Another type of vector is a viral vector (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), wherein additional DNA segments can be introduced into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. An "expression vector" is a type of vector that can direct the expression of a chosen polynucleotide.

A "host cell" is a cell that can be used to express a nucleic acid, e.g., a nucleic acid of the invention. A host cell can be a prokaryote, for example, E. coli, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), an animal cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman et al., 1981,
Cell 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (see Rasmussen et al., 1998, Cytotechnology 28:31) or CHO strain DX-B1 1, which is deficient in DHFR (see Urlaub et al., 1980, Proc. Natl. Acad. Sci. USA 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CVI/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMahan et al., 1991, EMBO J. 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Antigen binding proteins may be prepared by any of a number of conventional techniques. For example, they may be purified from cells that naturally express them (e.g., an antibody can be purified from a hybridoma that produces it), or produced in recombinant expression systems, using any technique known in the art. See, for example, Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Kennet et al. (eds.), Plenum Press, New York (1980); and Antibodies: A Laboratory Manual, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

Any expression system known in the art can be used to make the recombinant polypeptides of the invention. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example E. coli or bacilli. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, Cell 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163),

**0059** The transformed cells can be cultured under conditions that promote expression of the polypeptide, and the polypeptide recovered by conventional protein purification procedures. One such purification procedure includes the use of affinity chromatography. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian antibody polypeptides substantially free of contaminating endogenous materials.

**0060** Antigen binding proteins may be prepared, and screened for desired properties, by any of a number of known techniques. Certain of the techniques involve isolating a nucleic acid encoding a polypeptide chain (or portion thereof) of an antigen binding protein of interest, and manipulating the nucleic acid through recombinant DNA technology. The nucleic acid may be fused to another nucleic acid of interest, or altered (*e.g.*, by mutagenesis or other conventional techniques) to add, delete, or substitute one or more amino acid residues, for example.

**ANTIBODIES AND ANTIBODY FRAGMENTS**

**0061** The term "antibody" is used in the broadest sense and includes, for example, an intact immunoglobulin or to an antigen binding portion thereof that competes with the intact antibody for specific binding, unless otherwise specified. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include Fab, Fab′, F(αb′)2, Fd, Fv, and domain antibodies (dAbs), and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. Antibody includes a human antibody, a humanized antibody, chimeric antibody, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, an antigen-binding antibody fragment, a single chain antibody, a maxibody (scFv fused by a linker or direct attachment to an Fc or an Fe fragment), a diabody, a triabody, a tetrabody, a Fab fragment, an F(fa′)x fragment, a domain antibody, an IgD antibody, an IgE antibody, and IgM antibody, and IgGl antibody, and IgG2 antibody, and IgG3 antibody, and IgG4 antibody, and IgG4 antibody
having at least one mutation in the hinge region that alleviates a tendency to for intra H-chain disulfide bonds.

[0062] The term "polyclonal antibody" refers to a heterogeneous mixture of antibodies that bind to different epitopes of the same antigen.

[0063] The term "monoclonal antibodies" refers to a collection of antibodies encoded by the same nucleic acid molecule. In certain embodiments, monoclonal antibodies are produced by a single hybridoma or other cell line, or by a transgenic mammal. Monoclonal antibodies typically recognize the same epitope. The term "monoclonal" is not limited to any particular method for making an antibody.

[0064] A "Fab fragment" is a monovalent fragment having the V_L, V_H, C_L and C_H I domains; a F(ab')_2 fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the V_H and C_H I domains; an Fv fragment has the V_L and V_H domains of a single arm of an antibody; and a dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or V_L domain.

[0065] A "single-chain antibody" (scFv) is an antibody in which a V_L and a V_H region are joined via a linker (e.g., a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (see, e.g., Bird et al., 1988, Science 242:423-26 and Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-83). Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90:6444-48, and Poljak et al., 1994, Structure 2:1 121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

[0066] Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. An antigen
binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

[0067] The term "human antibody" includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). Human antibodies may be prepared in a variety of ways, including immunization of a mouse that is genetically modified to express human antibodies. One can engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce human antibodies in the absence of mouse antibodies. Large human Ig fragments may preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains may yield high affinity fully human antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human MAbs with the desired specificity may be produced and selected. Certain exemplary methods are described in WO 98/24893, U.S. Pat. No. 5,545,807, EP 546073B1, and EP 546073A1. Human antibodies can also be prepared by panning human antibody libraries expressed on phage, phagemids, ribosomes, or other particles.

[0068] A "humanized antibody" has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the
humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of methods for making humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

[0069] The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a particular species or isotype and the framework of another antibody of the same or different species or isotype.

[0070] Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification and using techniques well-known in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See, e.g., Bowie et al., 1991, Science 253:164.

[0071] The term "multispecific antibody" refers to an antibody wherein two or more variable regions bind to different epitopes. The epitopes may be on the same or different targets. In certain embodiments, a multispecific antibody is a "bispecific antibody," which recognizes two different epitopes on the same or different antigens.

[0072] Numerous methods of preparing bispecific antibodies are known in the art, and discussed in, e.g., U.S. Patent Application 09/839,632, filed April 20, 2001 (incorporated by reference herein). Such methods include the use of hybrid-hybridomas as described by Milstein et al., 1983, Nature 305:537, and others (U.S. Patent 4,474,893, U.S. Patent 6,106,833), and chemical coupling of antibody fragments (Brennan et al., 1985, Science 229:81; Glennie et al., 1987, J. Immunol. 139:2367; U.S. Patent 6,010,902). Moreover, bispecific antibodies can be produced via recombinant means, for example by using leucine zipper motifs (i.e., from the Fos and Jun proteins, which preferentially form heterodimers; Kostelny et al., 1992, J. Immunol. 148:1547) or other lock and key interactive domain structures as described in U.S. Patent 5,582,996. Additional useful techniques include those described in Kortt et al., 1997, supra; U.S. Patent 5,959,083; and U.S. Patent 5,807,706.

[0073] In the case of an antibody that binds a protein target, an "epitope" is the antigenic site on the protein that is recognized by the antibody, i.e., the minimum molecular
structure within the protein target to which the antibody binds. Epitopes on proteins may be
continuous (comprising a segment of continuous amino acids from the primary amino acid
sequence) or non-continuous (comprising amino acids that are not continuous in the primary
protein sequence but which are in close proximity in the three-dimensional folded protein).

Antibodies according to the invention will typically have a Kd in the range of
10⁻⁷ to 10⁻¹³ M; in some preferred embodiments the antibodies have a Kd of less than 10⁻⁹ M. The antibodies of the invention specifically bind to the disclosed fusion proteins with higher
affinity than they bind to other targets, including to the individual fusion partners SCNNIA
and TNFRSFIA. In some preferred embodiments the antibodies bind the SCNNIA 12/2
TNFRSFIA fusion protein with at least 10-fold higher affinity than they bind either of the
SCNNIA or TNFRSFIA fusion partners; in some preferred embodiments the antibodies bind
the SCNNIA 12/2 TNFRSFIA fusion protein with at least 100-fold higher affinity than they
bind either of the SCNNIA or TNFRSFIA fusion partners. In some preferred embodiments the
antibodies bind the SCNNIA 13/2 TNFRSFIA fusion protein with at least 10-fold higher
affinity than they bind either of the SCNNIA or TNFRSFIA fusion partners; in some preferred
embodiments the antibodies bind the SCNNIA 13/2 TNFRSFIA fusion protein with at least
100-fold higher affinity than they bind either of the SCNNIA or TNFRSFIA fusion partners.

In some embodiments of the invention a bispecific binding agent, e.g., a
bispecific antibody, recognizes the fusion proteins, with one antigen-binding site recognizing
SCNNIA and another antigen-binding site recognizing TNFRSFIA. In some embodiments,
one antigen-binding site recognizes the fusion protein (e.g., SCNNIA 12/2 TNFRSFIA or
SCNNIA 13/2 TNFRSFIA) and another antigen-binding site recognizes TNFRSFIA. In some
embodiments, one antigen-binding site recognizes the fusion protein (e.g., SCNNIA 12/2
TNFRSFIA or SCNNIA 13/2 TNFRSFIA) and another antigen-binding site recognizes
SCNNIA. In some embodiments one antigen-binding site recognizes the fusion protein and
another antigen-binding site recognizes another antigen such as, e.g., an antigen expressed on a
T-cell in order to leverage the cytotoxicity of T cells. In some embodiments the bispecific
antibodies have a lower affinity, e.g., a Kd of greater than 100 nM for each arm, in order to take
advantage of the avidity enhancement that can result from bispecific binding.

METHODS OF USING THE INVENTION TO IDENTIFY
AND CHARACTERIZE TUMORS

As illustrated in Example 3, the fusion proteins described herein are
differentially expressed in certain tumors, including ovarian, lung, thyroid, and bladder tumors,
as compared to normal tissues. Detecting expression and/or expression levels of the fusion proteins in tissue samples can therefore be used to identify a tumor, characterize a tumor, or to monitor the effects of treatment on tumors that express the identified fusion proteins. In some embodiments expression is detected at the RNA level, using methods described herein and known in the art such as Quantitative PCR, hybridization, in situ hybridization, nanostring technology (such as that described in U.S. Patent No. 7,473,767), and nucleic acid sequencing. In some embodiments expression is detected at the protein level, using methods described herein and known in the art such as immunoprecipitation, immunohistochemistry (IHC), Western blot analysis, flow cytometry, ELISA, immunoassays with antibody detection, and mass spectrometry.

METHODS OF USING THE INVENTION TO INHIBIT PROLIFERATION AND TREAT CANCER

[0077] The antibodies or other antigen binding agents of the invention may be used to inhibit proliferation of cells that express the identified fusion proteins, including for the treatment of ovarian, lung, bladder, thyroid, and other cancers.

[0078] In certain embodiments, the antibodies or other antigen binding agents are administered alone. In certain embodiments, the antibodies or other antigen binding agents are administered prior to the administration of at least one other therapeutic agent. In certain embodiments, the antibodies or other antigen binding agents are administered concurrent with the administration of at least one other therapeutic agent. In certain embodiments, the antibodies or other antigen binding agents are administered subsequent to the administration of at least one other therapeutic agent. Exemplary therapeutic agents include, but are not limited to, radiation therapy and chemotherapy.

[0079] The antibodies or other antigen binding agents are administered in a pharmaceutical composition including materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Such materials are non-toxic to the recipients at the dosages and concentrations employed, and include buffers such as TRIS HCl, phosphate, citrate, acetate and other organic acid salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidinone; amino acids such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives,
glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium and/or nonionic surfactants such as TWEEN, PLURONICS or polyethyleneglycol. The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. Delivery vehicles, diluents, excipients, and pharmaceutical adjuvants are known in the art and described in, e.g., Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company (1990).

[0080] The route of antibody administration is in accord with known methods, e.g., injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intrathecal, inhalation or intralesional routes, or by sustained release systems as noted below. Antibodies are preferably administered continuously by infusion or by bolus injection. An effective amount of antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. The dosage of the antibody formulation for a given patient will be determined by the attending physician taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors.

[0081] In some embodiments of the invention the antibodies or other antigen binding agents are able to directly modulate the function of the fusion proteins and therefore selectively kill tumor cells expressing those fusion proteins.

[0082] In some embodiments of the invention, the cell killing ability of antibodies is improved through conjugation to a cytotoxic agent or by enhancing an antibody effector function. These embodiments are particularly well-suited to killing cancer cells that express the fusion proteins of the invention. Antibodies with improved cell-killing ability, such as antibodies conjugated to a toxin or antibodies with enhanced effector function, can be used to kill tumor cells expressing the fusion proteins whether or not those fusion proteins are functional and whether or not the antibodies modulate that function. This is an important therapeutic advantage in the treatment of tumors.

[0083] The invention therefore includes compositions and use of "antibody-drug conjugates," or "ADCs" (which are also referred to immunoconjugates) comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate). The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit

[0084] In some embodiments of the invention the antibodies have an enhanced effector function. Antibody "effector function" refers to those biological activities attributable to the Fc region of an antibody, including complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors recognize bound antibody on a target cell and subsequently cause lysis of the target cell. In some embodiments, the ADCC activity of the antibodies has been enhanced through methods known in the art such as modification of the Fc sequence or modification of the carbohydrate structure, e.g., reducing fucose in the Fc-linked oligosaccharide structure of the antibodies to create "afucosylated antibodies."
EXAMPLES

EXAMPLE 1. SEQUENCING OVARIAN CANCER TRANSCRIPTOMES

[0085] RNA was extracted from eight samples: three ovarian serous adenocarcinoma stage 3 grade 3 tumors; three unmatched normal ovary; and two mixtures of multiple essential normal tissues, including colon, right atrium, liver, ventricle, esophagus, kidney, small intestine, lung, adrenal cortex, pituitary, pancreas, and stomach. The RNA was reverse transcribed and the resulting DNA sequenced to identify potential tumor-specific antigens. Using next generation sequencing, which can generate 100,000-fold or more sequence information as compared to capillary sequencing methods, yielded information about the identity and abundance of sequences within the transcriptomes. A comparison of sequences from the ovarian cancer samples as compared to the normal tissues identified differentially expressed genes, novel exons and splice junctions, and transcript fusions. Of approx. 7000 genes found to be overexpressed, 46 genes were upregulated at least 20-fold in ovarian tumors as compared to normal tissues and twelve were predicted to encode tumor-specific cell surface proteins. Of 350 transcript fusion candidates in which individual or paired sequence reads were mapped to two different genes, eight were tumor-specific and at least three were predicted to encode cell surface proteins. The transcriptomes of eight additional ovarian tumors as well as additional normal ovary, fallopian tube, and other normal tissues were also sequenced and two ovarian cancer-specific fusion proteins were further characterized as described in Examples 2 and 3:

EXAMPLE 2. IDENTIFICATION AND SEQUENCING OF OVARIAN CANCER FUSION PROTEINS

[0086] Two fusion transcripts between adjacent genes SCNN1A (NCBI Reference Sequence: NM_001038.5) and LNFRSF1A (NCBI Reference Sequence: NM_001065.2) were specifically found in ovarian tumors vs. normal tissues and were predicted to encode cell surface proteins. Using cDNA prepared from RNA from ovarian tumors and ovarian cancer cell lines, the fusion junctions were confirmed. Additional characterization of the fusion transcripts by RACE-PCR (rapid amplification of cDNA ends polymerase chain reaction) revealed multiple potential full length fusion cDNA sequences, all of which contained the fusion junctions as described below.

[0087] The SCNN1A 12/2 LNFRSF1A fusion ("the 12/2 fusion") is believed to result from tumor-specific splicing between exon 12 of SCNN1A and exon 2 of LNFRSF1A, and

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includes the fusion junction peptide sequence TNSESPSVT-VLLELLVGIY (SEQ ID NO: 1; the "-" shows the point of fusion). A predicted full length cDNA sequence corresponding to a fused SCNN1A 12/2 TNFRSF1A transcript is shown as SEQ ID NO: 3. The deduced amino acid sequence of the fusion protein is shown as SEQ ID NO: 4. The fusion junction peptide is shown as residues 535-553 of SEQ ID NO: 4.

[0088] The SCNN1A 13/2 TNFRSF1A fusion ("the 13/2 fusion") is believed to result from tumor-specific splicing between exon 13 of SCNN1A and exon 2 of TNFRSF1A, and includes the fusion junction peptide sequence GRGGRGAQE-VLLELLVGIY (SEQ ID NO: 2; the "-" shows the point of fusion). A predicted full length cDNA sequence corresponding to a fused SCNN1A 13/2 TNFRSF1A transcript is shown as SEQ ID NO: 5. The deduced amino acid sequence of the fusion protein is shown as SEQ ID NO: 6. The fusion junction peptide is shown as residues 596-614 of SEQ ID NO: 6.

[0089] SCNN1A-TNFRSF1A fusions were observed in all eleven ovarian tumors sequenced.

[0090] SCNN1A occurs in different isoforms, including NM_001038.5/NP_001029.1, NM_001159576.1/NP_001153048.1, and NM_001159575.1/NP_001153047.1, and tumor-specific splicing can involve any of these isoforms. In some fusion proteins, therefore, the deduced amino acid sequence of the fusion protein has an additional 59 amino acids (MGMARGSLTR VPGVMGEQT GPELSLPDP CSPQSTPGLM KGNKLEEQDP RPLQPIPGL, SEQ ID NO: 13) at the N-terminus. In other fusion proteins, the deduced amino acid sequence of the fusion protein has an additional 23 amino acids, (MSSIKGNKLE EQDPRPLQPI PGL, SEQ ID NO: 14) at the N-terminus.

EXAMPLE 3. SCNN1A-TNFRSF1A FUSION PROTEIN EXPRESSION

[0091] Quantitative RT-PCR methods were developed to specifically detect each fusion. For the 12/2 fusion, the junction site was amplified by primers 5'-CATCTTTCTTAAG GAGCTGAACTACA-3' (SEQ ID NO: 7) and 5'-GACCAGTCC AATAACCCCTGAG-3' (SEQ ID NO: 8), and detected with a fluorescently-labeled TaqMan® (Real-Time PCR, Applied Biosystems) hydrolysis probe 5'-6FAM-CTGTCACGCTGCTCT-MGB-3' (SEQ ID NO: 9, where 6FAM and MGB are a 6-carboxyfluorescein fluorophore and a dihydrocyclopyrroloindole tripeptide minor groove binder quencher respectively) covering the junction site.
For the 13/2 fusion, the junction site was amplified by primers 5'-CCGAAGCCGATACTGGTCTC-3' (SEQ ID NO: 10) and 5'-GACCAGTCCATAACCCCCTGAG-3' (SEQ ID NO: 11), and detected with a fluorescently-labeled TaqMan® (Real-Time PCR, Applied Biosystems) hydrolysis probe 5'-6FAM-CTCAGGAGGTGCTCCT-MGB-3' (SEQ ID NO: 12, where 6FAM and MGB are a 6-carboxyfluorescein fluorophore and a dihydrocyclopypyrrolomdole tripeptide minor groove binder quencher respectively) covering the junction site.

As shown in Figures 1 and 2, quantitative PCR analysis confirmed that SCNN1A-TNFRSF1A fusion proteins are preferentially expressed in ovarian cancer tissue as compared to normal tissues. Quantitative PCR also detected differential expression of the fusions in lung, thyroid, and bladder tumors as compared to normal tissues. Albeit low, there was detectable expression of the fusions in some normal tissues, such as lung and kidney.

Immunoprecipitation and Western Blot analysis showed that cancer cell lines which had been identified as expressing fusion messages also express fusion proteins. A commercially available anti-TNFR antibody was used to immunoprecipitate protein from cell lysates which were then analyzed by Western Blot using a commercially available anti-SCNN1A antibody. This revealed the presence of a band near the estimated MW of the fusion proteins in cell lines positive for fusion message that was absent in those cell lines negative for fusion message.

Additionally, mass spectrometric identification of peptides from both fusion partners (SCNNIA and TNFRSF1A) was obtained by analyzing the protein in the gel excised at the fusion protein band location (neither wildtype protein migrates at this location). The ratio of SI(N) (Normalized Spectral Index) values calculated for the fusion partners were consistent with a 1:1 stoichiometry between SCNNIA and TNFRSF1A, further substantiating expression of the fusion proteins.

EXAMPLE 4. ANTIBODIES TO FUSION PEPTIDES AND FUSION PROTEINS

The fusion junction peptides of SEQ ID NOs: 1 and 2 were separately synthesized and used as antigens to make polyclonal or monoclonal antibodies using standard techniques known in the art.

Longer fragments containing the junction peptides were also used as antigens to generate antibodies, e.g., for the 12/2 fusion ELNYKTNSESPTVLLVGIYPSGVIG
(residues 530-559 of SEQ ID NO: 4) and for the 13/2 fusion RYWSPGRGGRGAQEVLLL
ELLVGIYPSGVIG (residues 591-620 of SEQ ID NO: 6).

[0098] Fragments containing the junction peptides and predicted to include extracellular domains are also used as antigens to generate antibodies, e.g., for the 12/2 fusion residues 108-741 of SEQ ID NO: 4 and for the 13/2 fusion residues 108-802 of SEQ ID NO: 6.

[0099] Additional antibodies are similarly made to the fusion proteins shown in SEQ ID NOs: 4 and 6 and to the fusion proteins and fragments thereof that are expressed on a recombinant host cell.

[0100] The antibodies generated against the fusion junction peptides, fusion proteins, and expressed fusion proteins are used to confirm protein expression and study protein localization in ovarian and other tumors, to detect and characterize ovarian and other tumors, and to treat ovarian cancer and other cancers including lung cancer, thyroid cancer, and bladder cancer.

[0101] Human antibodies that specifically bind to the SCNN1A-TNFRSF 1A fusion proteins are particularly useful for treating cancers including lung cancer, thyroid cancer, and bladder cancer. Human antibodies that specifically bind to the SCNN1A-TNFRSF 1A fusion proteins and are conjugated to a cytotoxic agent and/or modified to have an enhanced effector function are particularly useful for treating cancers including lung cancer, thyroid cancer, and bladder cancer.
What is claimed:

1. An isolated antigen binding agent that specifically binds to a SCNNIA /TNFRSFIA fusion protein.

2. The antigen binding agent of claim 1, wherein the antigen binding agent specifically binds to a SCNNIA /TNFRSFIA fusion protein selected from:
   (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1, and
   (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2.

3. The antigen binding agent of claim 1 or claim 2, wherein the antigen binding agent is a monoclonal antibody.

4. The monoclonal antibody of claim 3, wherein the antibody binds the SCNNIA /TNFRSFIA fusion protein with an affinity less than 1 nM and specifically binds the SCNNIA /TNFRSFIA fusion protein with at least 100-fold higher affinity than it binds either SCNNIA or TNFRSFIA.

5. The antibody of claim 3 or claim 4, wherein the antibody is conjugated to a cytotoxic agent.

6. The antibody of claim 5, wherein the cytotoxic agent is an auristatin or maytansinoid.

7. The antibody of claim 3 or claim 4, wherein the antibody has an enhanced effector function.

8. The antigen binding agent of claim 1, wherein the antigen binding agent is a bispecific antibody in which one antigen-binding site binds an epitope on SCNNIA and one antigen-binding site binds an epitope on TNFRSFIA.
9. The bispecific antibody of claim 8, wherein the antibody specifically binds to a SCNN1A/TNFRSF1A fusion protein selected from:
   (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1, and
   (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2.

10. A method of identifying or characterizing a tumor comprising detecting expression in cells from the tumor of a fusion protein selected from:
    (a) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1, and
    (b) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2.

11. The method of claim 10, wherein expression of the fusion protein is detected at the level of RNA.

12. The method of claim 10, wherein expression of the fusion protein is detected at the level of protein.

13. The method of claim 12, wherein expression of the fusion protein is detected through the use of an antibody that specifically binds the fusion protein.

14. A method of inhibiting proliferation of cells expressing a SCNN1A/TNFRSF1A fusion protein comprising contacting the cells with a composition comprising an antigen binding agent or bispecific antibody according to one of claims 1-9.

15. A method of inhibiting proliferation of cells expressing a SCNN1A/TNFRSF1A fusion protein comprising contacting the cells with a composition comprising an antibody according to one of claims 6 or 7.

16. A method of treating a patient with cancer, comprising administering a composition comprising an antigen binding agent or bispecific antibody according to one of claims 1-9.

17. A method of treating a patient with cancer, comprising administering a composition comprising an antibody according to one of claims 6 or 7.
18. The method of claim 16 or claim 17, wherein the cancer is selected from ovarian cancer, bladder cancer, thyroid cancer, and lung cancer.

19. The method of one of claims 16-18, wherein treatment is administered after detection of the expression of a SCNNIA/TNFRSFIA fusion protein in cells from the cancer.

20. A method of treating a patient with cancer comprising the steps of:
   (a) detecting expression of a SCNNIA/TNFRSFIA fusion protein in cells from the cancer, and
   (b) administering an antibody according to one of claims 6 or 7.
AMENDED CLAIMS
received by the International Bureau on 16 December 2011 (16.12.2011)

CLAIMS

What is claimed:

1. An isolated antigen binding agent that specifically binds to a SCNNIA /TNFRSFIA fusion protein.

2. The antigen binding agent of claim 1, wherein the antigen binding agent specifically binds to a SCNNIA /TNFRSFIA fusion protein selected from:
   (a) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2, and
   (b) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1.

3. The antigen binding agent of claim 1 or claim 2, wherein the antigen binding agent is a monoclonal antibody.

4. The monoclonal antibody of claim 3, wherein the antibody binds the SCNNIA /TNFRSFIA fusion protein with an affinity less than 1 nM and specifically binds the SCNNIA /TNFRSFIA fusion protein with at least 100-fold higher affinity than it binds either SCNNIA or TNFRSFIA.

5. The antibody of claim 3 or claim 4, wherein the antibody is conjugated to a cytotoxic agent.

6. The antibody of claim 5, wherein the cytotoxic agent is an auristatin or maytansinoid.

7. The antibody of claim 3 or claim 4, wherein the antibody has an enhanced effector function.

8. The antigen binding agent of claim 1, wherein the antigen binding agent is a bispecific antibody in which one antigen-binding site binds an epitope on SCNNIA and one antigen-binding site binds an epitope on TNFRSFIA.
9. The bispecific antibody of claim 8, wherein the antibody specifically binds to a SCNN1A/TNFRSF1A fusion protein selected from:
   (a) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2, and
   (b) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1.

10. A method of identifying or characterizing a tumor comprising detecting expression in cells from the tumor of a fusion protein selected from:
    (a) SEQ ID NO: 6, or a fragment thereof comprising the sequence SEQ ID NO: 2, and
    (b) SEQ ID NO: 4, or a fragment thereof comprising the sequence SEQ ID NO: 1.

11. The method of claim 10, wherein expression of the fusion protein is detected at the level of RNA.

12. The method of claim 10, wherein expression of the fusion protein is detected at the level of protein.

13. The method of claim 12, wherein expression of the fusion protein is detected through the use of an antibody that specifically binds the fusion protein.

14. A method of inhibiting proliferation of cells expressing a SCNN1A/TNFRSF1A fusion protein comprising contacting the cells with a composition comprising an antigen binding agent or bispecific antibody according to one of claims 1-9.

15. A method of inhibiting proliferation of cells expressing a SCNN1A/TNFRSF1A fusion protein comprising contacting the cells with a composition comprising an antibody according to one of claims 6 or 7.

16. A method of treating a patient with cancer, comprising administering a composition comprising an antigen binding agent or bispecific antibody according to one of claims 1-9.

17. A method of treating a patient with cancer, comprising administering a composition comprising an antibody according to one of claims 6 or 7.
18. The method of claim 16 or claim 17, wherein the cancer is selected from ovarian cancer, bladder cancer, thyroid cancer, and lung cancer.

19. The method of one of claims 16-18, wherein treatment is administered after detection of the expression of a SCNNIA /TNFRSFIA fusion protein in cells from the cancer.

20. A method of treating a patient with cancer comprising the steps of:
   (a) detecting expression of a SCNNIA /TNFRSFIA fusion protein in cells from the cancer, and
   (b) administering an antibody according to one of claims 6 or 7.
Fig. 1
INTERNATIONAL SEARCH REPORT

According to International Patent Classification (IPC) or to both national classification and IPC

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>wo 2008/093323 A2 (COMPUGEN LTD.) 7 August 2008 (2008-08-07) 702 A1</td>
<td>1, 2</td>
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<td>Y</td>
<td>page 156, line 16 - page 159, line 2 cl aim 19 table 99 sequence 102</td>
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<td>U. CHRISTEN ET AL.: &quot;Immune response to a recombinant human TNFR55-1gGl fusion protein: Auto-anti body responses in rheumatoid arthritis (RA) and multiple sclerosis (MS) patients have neither neutralizing nor agonistic activities. &quot;, HUMAN IMMUNOLOGY, vol. 60, no. 9, September 1999 (1999-09), pages 774-790, XP002314027, USA</td>
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<td>Y</td>
<td>abstract</td>
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Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
  * "A" document defining the general state of the art which is not considered to be of particular relevance
  * "B" earlier document published on or after the international filing date
  * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  * "O" document referring to an oral disclosure, use, exhibition or other means
  * "P" document published prior to the international filing date but later than the priority date claimed
  * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  * "Z" document member of the same patent family

Date of the actual completion of the international search: 14 October 2011
Date of mailing of the international search report: 20/10/2011

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentliaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-0040, Fax: (+31-70) 340-0016

Authorized officer: Nooij, Frans
## DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>page 129, left-hand col umn, paragraph 3 - right-hand col umn, paragraph 2</td>
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<td>F. STANKE ET AL.: &quot;The TNFalpha receptor TNFRSF1A and genes encoding the amiloride-sensitive sodium channel ENaC as modulators in cystic fibrosis s.&quot;, HUMAN GENETICS, vol. 119, no. 3, April 2006 (2006-04), pages 331-343, XP000002655588, Germany</td>
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Form PCT/ISA/210 (continuation of second sheet) (April 2009)
INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2.☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2.☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 

4.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: l-20(partly)

An isolated antigen binding agent that specifically binds to a SCNN1A/TNFRSF1A fusion protein; a method of identifying or characterizing a tumor comprising detecting expression in cells from the tumor of a fusion protein; a method of inhibiting proliferation of cells expressing a SCNN1A/TNFRSF1A fusion protein comprising contacting the cell with a composition comprising said binding agent; a method of treating a patient with cancer comprising administering a composition comprising said binding agent, the above in which said SCNN1A/TNFRSF1A fusion protein is selected from SEQ ID NO:4, or a fragment thereof comprising the sequence of SEQ ID NO:1.

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2. claims: l-20(partly)

An isolated antigen binding agent that specifically binds to a SCNN1A/TNFRSF1A fusion protein; a method of identifying or characterizing a tumor comprising detecting expression in cells from the tumor of a fusion protein; a method of inhibiting proliferation of cells expressing a SCNN1A/TNFRSF1A fusion protein comprising contacting the cell with a composition comprising said binding agent; a method of treating a patient with cancer comprising administering a composition comprising said binding agent, the above in which said SCNN1A/TNFRSF1A fusion protein is selected from SEQ ID NO:6, or a fragment thereof comprising the sequence of SEQ ID NO:2.

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